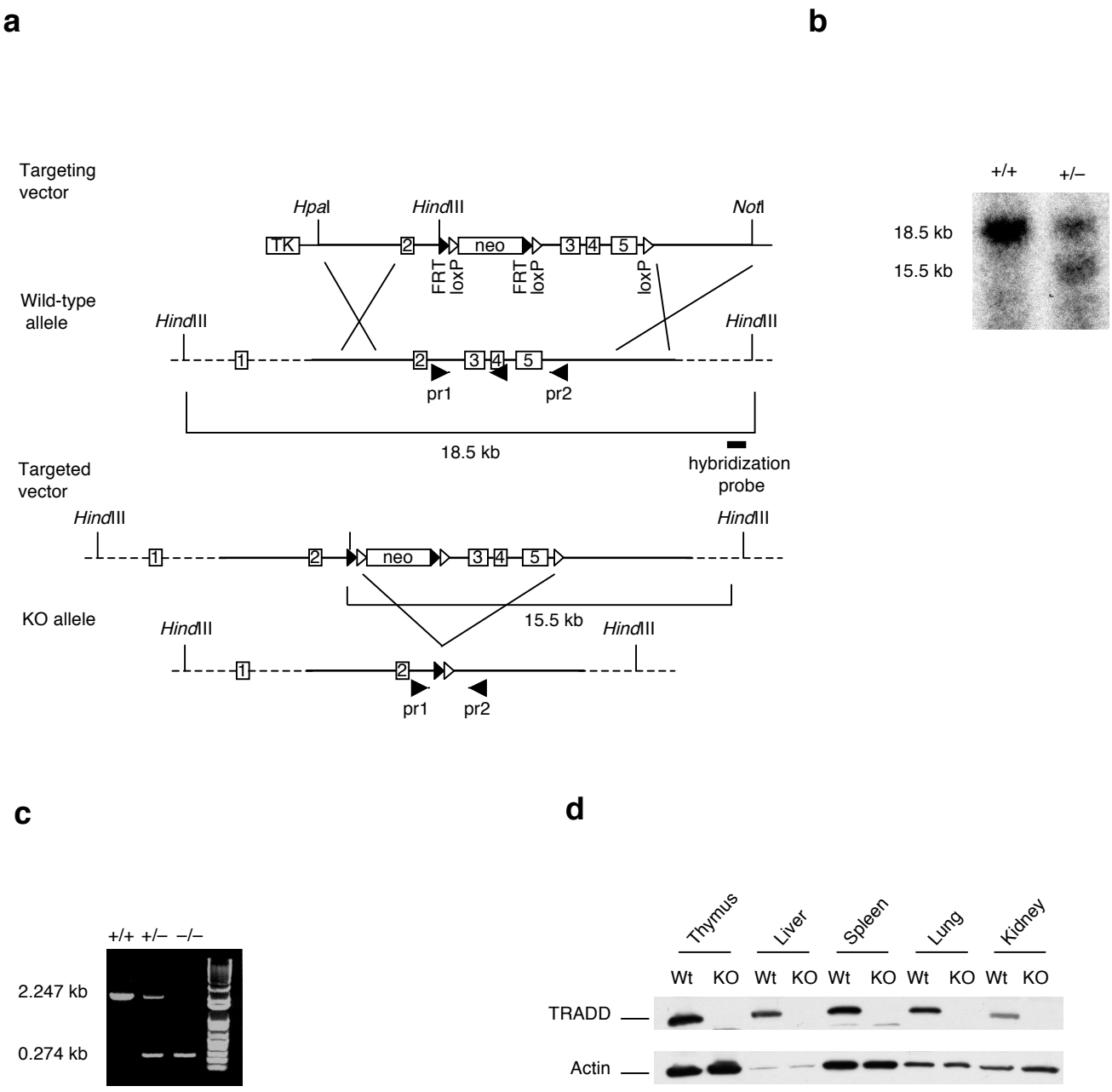
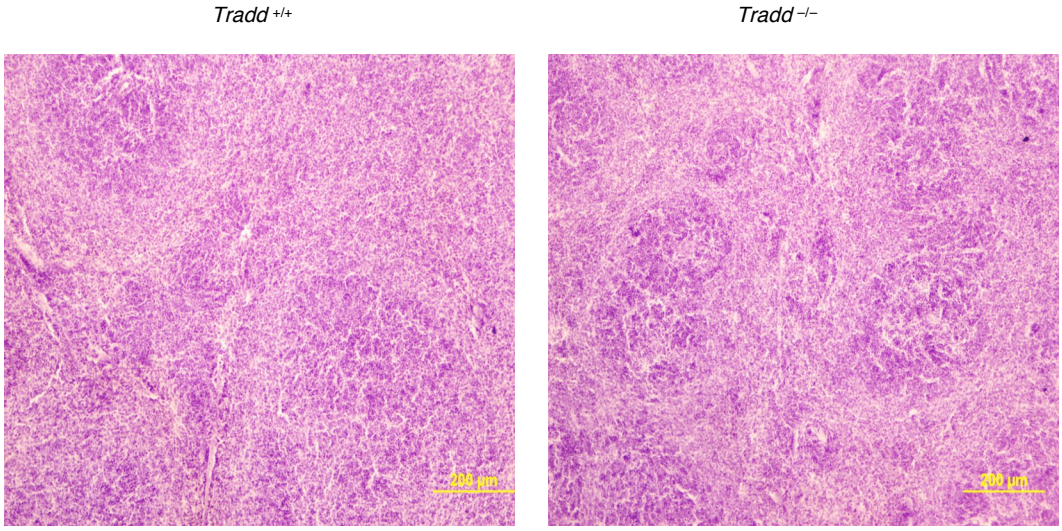


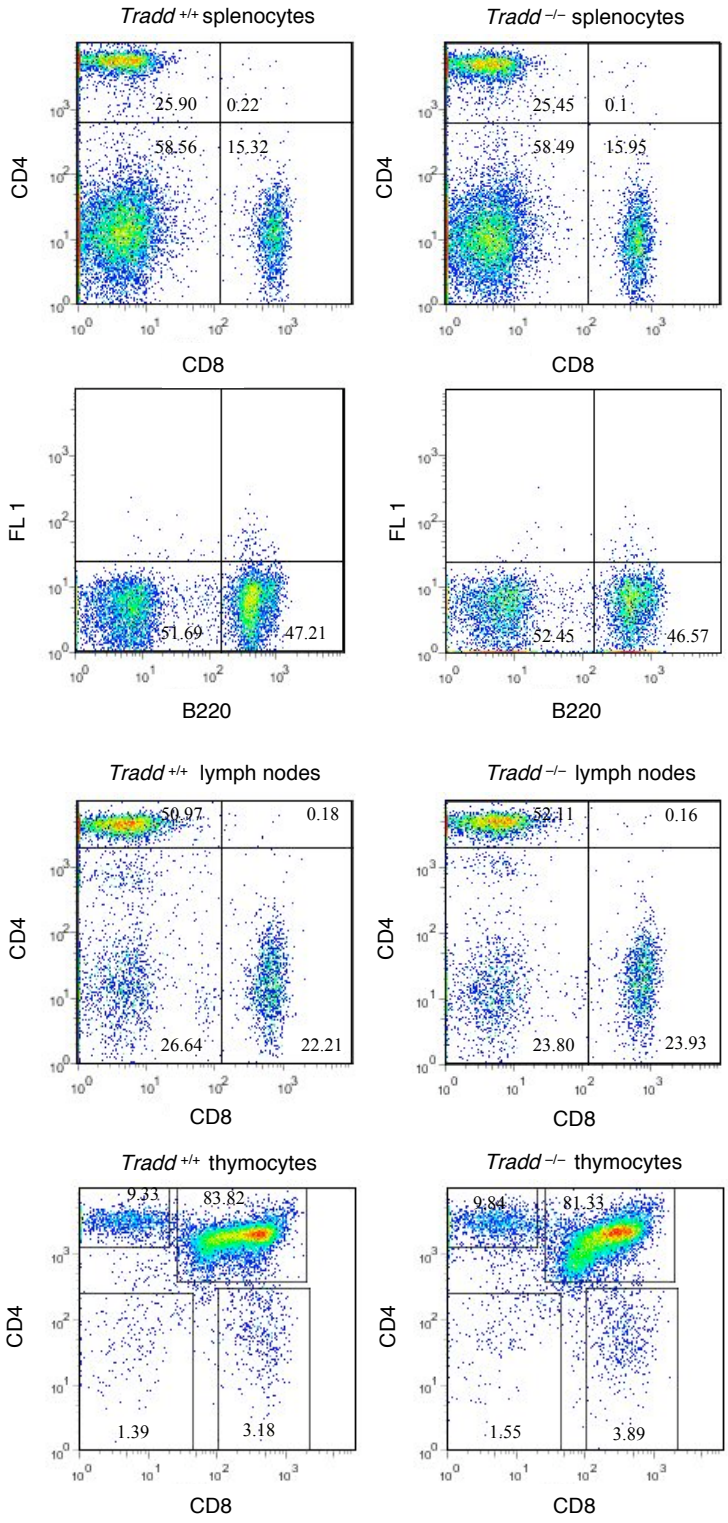
Supplementary Figure 1.*Tradd* gene targeting and deletion. (**a**) Scheme of the targeted disruption of the *Tradd* gene. Wild-type, targeted and mutant loci are shown. Cre-mediated recombination *in vivo* creates a deletion of a 4 kb fragment that includes the 1.9 kb *neo*^{r} cassette and a 2 kb genomic segment containing *Tradd* exons 3, 4 and 5. Locations of the hybridization probe and PCR primers (pr1, pr2) used to verify deletion are indicated. (**b**) Southern blot analysis of ES cell DNA. (**c**) PCR analysis of tail DNA of wild-type, heterozygous and knockout mice. (**d**) Immunoblot analysis of different tissues from wild-type and knockout (KO) mice.

Genotype	Number of mice	PP per mouse
Tradd $^{+/+}$	7	7-8
Tradd $^{-/-}$	10	6-7
Tnfr1 $^{-/-}$	3	2-3

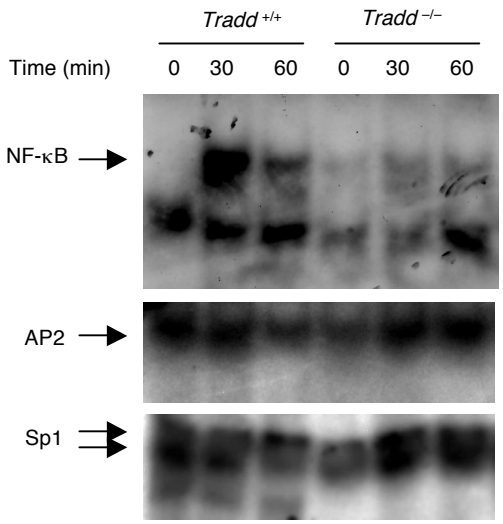
Supplementary Table 1. Analysis of Peyer's patches (PP) in *Tradd* $^{+/+}$, *Tradd* $^{-/-}$ and *Tnfr1* $^{-/-}$ mice. Mice were sacrificed, intestines were removed, placed in cold PBS and PP were counted. Average number of PP per mouse is shown.



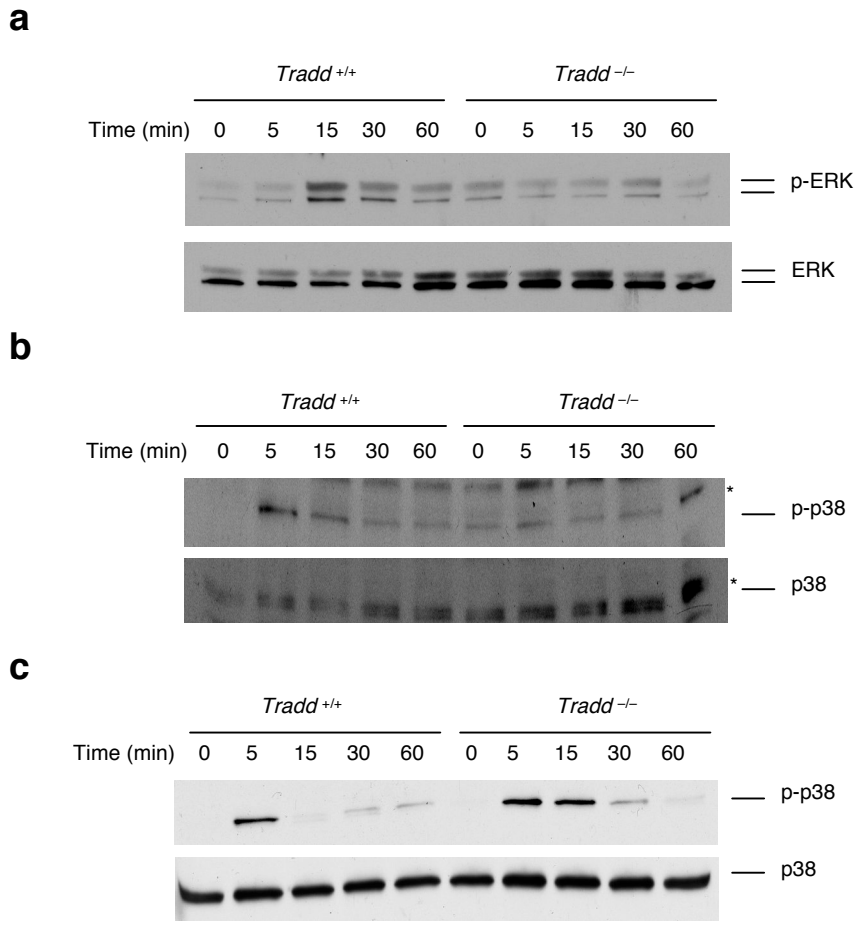
Supplementary Figure 2. Histological analysis of spleens from *Tradd* $^{+/+}$ and *Tradd* $^{-/-}$ mice. Spleens were excised, fixed in formalin and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin. Representative spleen sections of *Tradd* $^{+/+}$ and *Tradd* $^{-/-}$ mice are shown. Data are representative of independent staining of two mice.



Supplementary Figure 3. Composition of lymphocyte subsets in *Tradd* $^{+/+}$ and *Tradd* $^{-/-}$ mice. Lymphocytes from spleens, lymph nodes and thymuses were stained with anti-CD4, anti-CD8 and anti-B220 (splenocytes only) and analyzed by flow cytometry. Data are representative of independent staining of 4 mice.

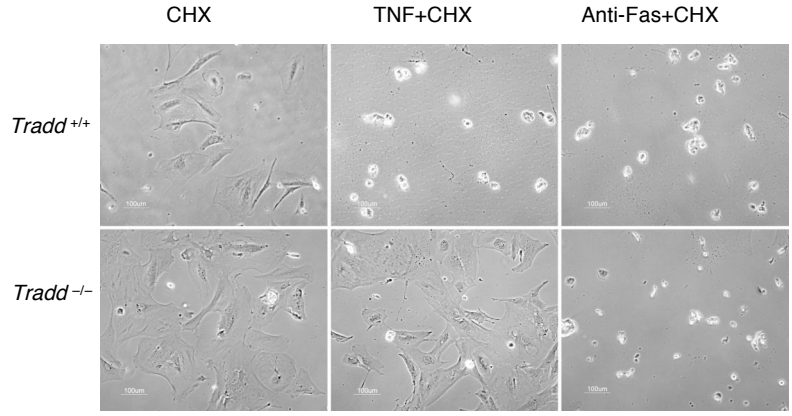


Supplementary Figure 4 . NF-kB does not bind DNA after TNF treatment in *Tradd* $^{-/-}$ MEFs. Nuclear extracts were isolated from MEFs that were stimulated for the indicated periods with TNF. Gel shifts were performed as indicated in Methods. Data represents 2 independent experiments.

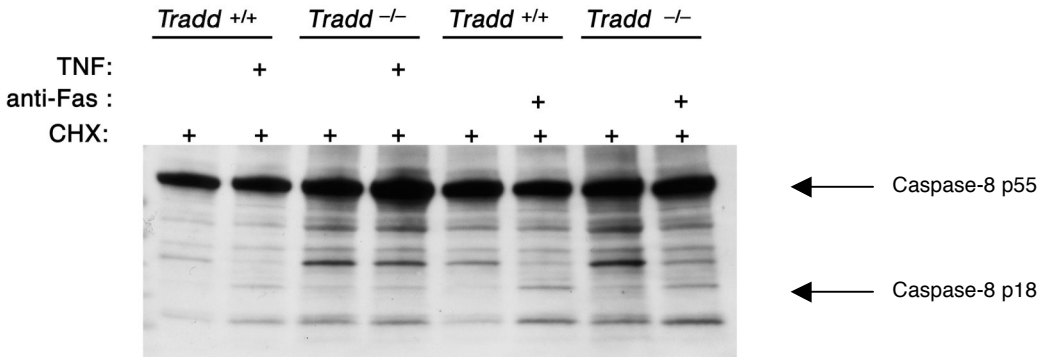


Supplementary Figure 5. TRADD is required for TNF but not IL-1-induced ERK and p38 activation in MEFs. Wild-type and *Tradd* $^{-/-}$ cells were treated with TNF (**a,b**) (30ng/ml) or IL-1b (**c**) (20ng/ml) for indicated time periods and cell extracts were applied to SDS-PAGE for immunoblotting with indicated antibodies. *appearance of stronger band in the last lane due to the marker. Data represents three independent experiments.

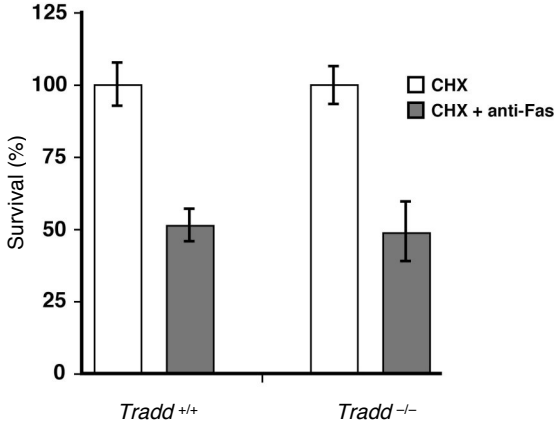
a



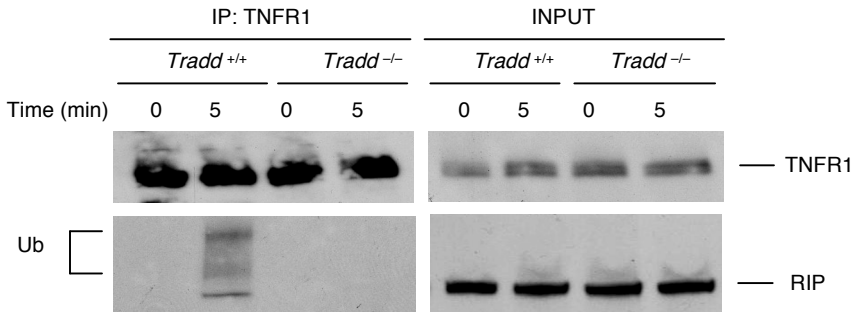
b



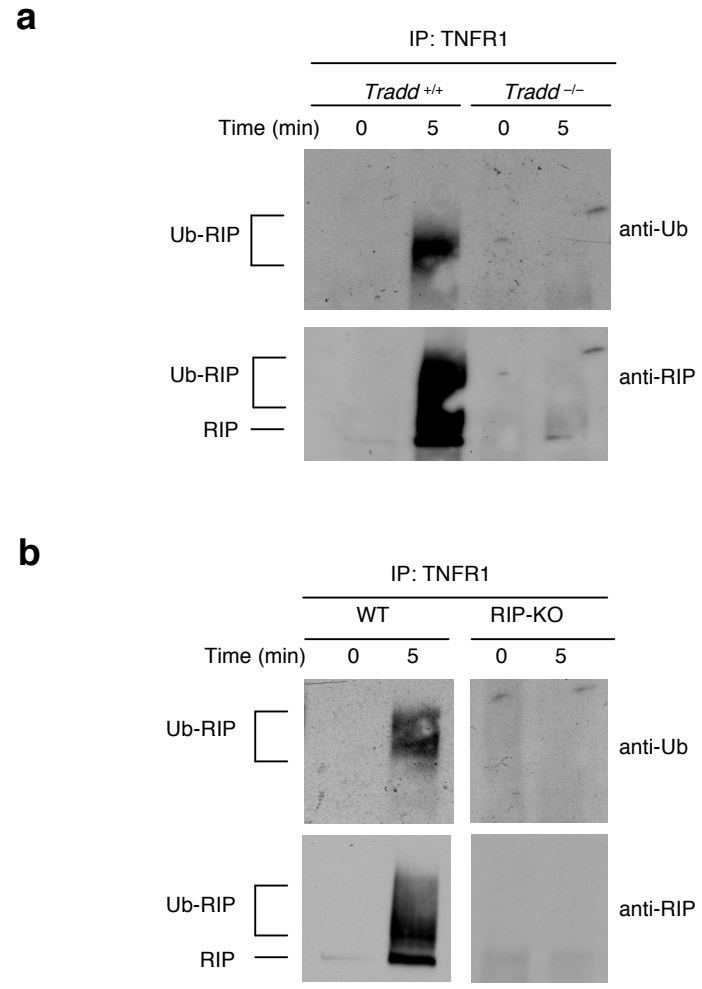
c



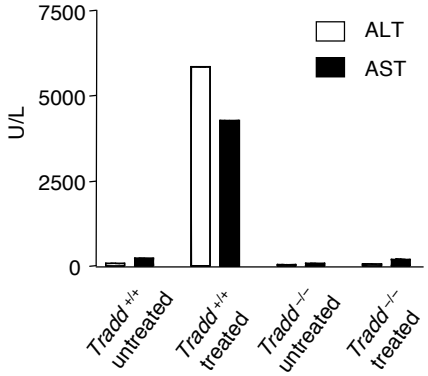
Supplementary Figure 6. TRADD is not required for Fas-mediated apoptosis. Wild-type and *Tradd* $^{-/-}$ MEFs (**a,b**) and thymocytes (**c**) were treated with TNF (30ng/ml) plus CHX (10mg/ml) or anti-Fas (10mg/ml) plus CHX for 24 hours and analyzed by phase-contrast microscopy (**a**), by immunoblotting with anti-caspase8 (**b**) or by MTS assay (error bars ± s.e.m.) (**c**). Data represents three independent experiments.



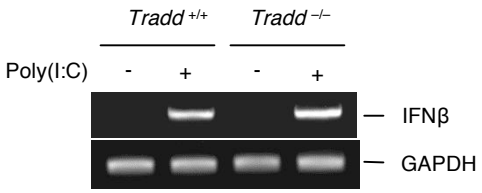
Supplementary Figure 7. Similar amounts of TNFR1 are immunoprecipitated from *Tradd* $^{+/+}$ and *Tradd* $^{-/-}$ MEFs. Cells were stimulated with TNF for the indicated periods, TNFR1 was immunoprecipitated with a TNFR1-specific antibody coupled to protein G-Agarose beads with dimethyl pimelimidate (DMP), and blots were probed with indicated antibodies. Data represents three independent experiments.



Supplementary Figure 8. Modification of RIP is due to ubiquitination. Wild-type, *Tradd* $^{-/-}$ and RIP-KO MEF cells were treated with TNF (30ng/ml) for the indicated time periods and protein lysates were immunoprecipitated with a TNFR1-specific antibody and analyzed by immunoblotting with indicated antibodies. Data represents three independent experiments.



Supplementary Figure 9. Serum concentrations of liver enzymes at 6 hours after GalN + LPS administration. Results are averages ± s.d. (n=8 for *Tradd* $^{+/+}$ mice, n=6 for *Tradd* $^{-/-}$ mice).



Supplementary Figure 10. Wild-type and *Tradd* $^{-/-}$ macrophages express similar amounts of IFNβ mRNA in response to Poly(I:C). Peritoneal macrophages were treated with 100mg/ml of Poly(I:C) for 24 hours. Total RNA was extracted and IFNβ mRNA was measured. Data represents three independent experiments.

Supplementary Methods

Gel shift assay. Nuclear extracts were isolated using the Biovision Nuclear/Cytosol fractionation kit following the manufactures guidlines. Gel shifts were performed with the Promega Gel shift assay system using 5 mg of nuclear extract and following the recommendations outlined in the manufacturer's protocol. All consensus oligos were also purchased from Promega.

RNA isolation. Total RNA was extracted using TRIZOL (Invitrigen) according to manufacturer's protocol.

Reverse transcriptase-mediated semiquantitative PCR. Total relative levels of IFNβ mRNA were determined by RT-PCR using TITANIUM one-step RT-PCR kit (Clontech). Sequences of the primers for IFNβ were published previously[1].

1. Alexopoulou, L., Holt, A.C., Medzhitov, R. & Flavell, R.A. Recognition of double stranded RNA and activation of NFkB by Toll-like receptor 3. *Nature* **413**, 732–738 (2001).